P28460.A05

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Kazutoshi WATANABE et al.

Group Art Unit : 1624

Appln. No : 10/550,299

Examiner : Balasubramanian

I.A. Filed : March 26, 2004

Confirmation No. 1935

For : 2,3,6-TRISUBSTITUTED-4-PYRIMIDONE DERIVATIVES

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
U.S. Patent and Trademark Office
Customer Service Window, Mail Stop **Amendment**
Randolph Building
401 Dulany Street
Alexandria VA 22314

Sir :

I, the undersigned, Dr. Kazutoshi Watanabe, a citizen of Japan, do solemnly declare as follows:

1. That I graduated from the Graduate School of Science (Chemistry), University of Tokyo, receiving a master degree in 1988. I also received a PhD from the Graduate School of Engineering, University of Tokyo, in 2003, an M.S. from the Graduate School of Science (Chemistry), University of Tokyo in 1988, and a B.S. from the Faculty of Science (Chemistry), University of Tokyo in 1986. I have specialized in the field of chemistry and have engaged in research in the chemical field for approximately twenty years. I have been an employee of companies that are associated with Mitsubishi Pharma Corporation since 1988 and am currently employed by Mitsubishi Pharma Corporation in the Pharmaceuticals Research Division.

2. That the following is a list of articles of which I am a coauthor in the field of art relevant to the above-reference application:

"Antioxidant activity of 3-methyl-1-phenyl-2-pyrazolin-5-one," Yorihiro Yamamoto, Tomohiro Kuwahara, **Kazutoshi Watanabe**, and Kazuhiko Watanabe, Redox Report 2:333-338 (1996);

"Radical scavenging mechanism of MCI-186," **Kazutoshi Watanabe**, Kazuhiko Watanabe, and Tetsuo Hayase, Jpn. Pharmacol. Ther. 25(Suppl. 7):189-197 (1997);

"Chemical, pharmacological and clinical profile of a neuroprotective agent ederavone," **Kazutoshi Watanabe** and Masahiko Tanaka, Pharmacometrics 65:79-88 (2003);

"Research and development of the free radical scavenger edaravone as a neuroprotectant," Toshiaki Watanabe, Masahiko Tanaka, **Kazutoshi Watanabe**, Yasuo Takamatsu, and Akihiro Tobe, Yakugaku Zasshi 124(3):99-111 (2004);

"Synthesis of the metabolites of a free radical scavenger edaravone (MCI-186, Radicut)," **Kazutoshi Watanabe**, Masao Taniguchi, and Masaki Shinoda, Redox Report 8(3):157-161 (2003);

"Structure-activity relationship of 3-methyl-1-phenyl-2-pyrazolin-5-one (edaravone)," **Kazutoshi Watanabe**, Yasuhiro Morinaka, Katsuhiko Iseki, Toshiaki Watanabe, Satoshi Yuki, and Hiroyoshi Nishi, Redox Report 8(3):151-155 (2003);

"Free radical-induced oxidation products of 3-methyl-1-phenyl-2-pyrazolin-5-one (MCI-186)," **Kazutoshi Watanabe**, Kazuhiko Watanabe, Tomohiro Kuwahara, and Yorihiro Yamamoto, Nihon Yukagakkaishi 46(7):797-801 (1997);

"Pharmacokinetic studies of 3-methyl-1-phenyl-2-pyrazolin-5-one (MCI-186): Metabolism in rats, dogs and human," Teiko Komatsu, Hiroshi Nakai, Yasuo Takamatsu, Yasuhiro Morinaka, **Kazutoshi Watanabe**, Masaki Shinoda, and Seiu Iida, Yakubutsu Dotai 11(5):451-462 (1996);

"Generation of Lithium Enolates Accelerated by Lithium Trifluoromethanesulfonate: Application to the Selective 1,4-Chiral Induction in the Aldol Reaction of *t*-Butyl δ-Hydroxy Carboxylates," K. Narasaka, Y. Ukaji, and **K. Watanabe**, Chem. Lett. 1755-1758 (1986); and

"Selective 1,4-Chiral Induction in the Reaction of Enolates Generated from *t*-Butyl δ - Hydroxy Carboxylates," K. Narasaka, Y. Ukaji, and K. Watanabe, Bull. Chem. Soc. Jpn. 60:1457-1464 (1987).

3. That I am one of the inventors of the above-referenced application.

4. That I have reviewed the Office Action mailed October 19, 2006.

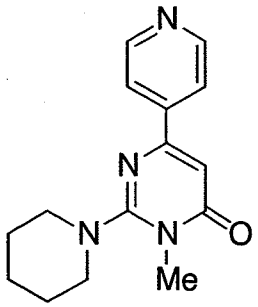
5. That experiments have been conducted under my direction to show the inhibitory activity of Compound Nos. 38 and 39 of EP 1136482 A1 to Almario-Garcia, as compared to compounds of the present invention as listed in Table-1 attached hereto, against P-GS1 phosphorylation by bovine cerebral TPK1.

6. That the experiments for measuring the inhibitory activity of Compound Nos. 38 and 39 of EP 1136482 A1 to Almario-Garcia, as compared to compounds of the present invention as listed in the attached Table-1, against P-GS1 phosphorylation by bovine cerebral TPK1 were performed as follows:

A mixture containing 100 mM MES-sodium hydroxide (pH 6. 5), 1 mM magnesium acetate, 0. 5 mM EGTA, 5 mM a-mercaptoethanol, 0.02% Tween 20, 10% glycerol, 12 μg/ml P-GS1, 41.7 μM[γ-^{32}P] ATP (68 kBq/ml), bovine cerebral TPK1 and a compound shown in the attached Table-1 or Compound No. 38 or Compound No. 39 of EP 1136482 A1 to Almario-Garcia (a final mixture contained 1.7% DMSO deriving from a solution of a test compound prepared in the presence of 10% DMSO) was used as a reaction system. The phosphorylation was started by adding ATP, and the reaction was conducted at 25°C for 2 hours, and then stopped by adding 21%

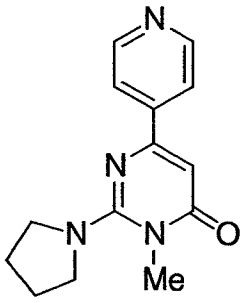
perchloric acid on ice cooling. The reaction mixture was centrifuged at 12,000 rpm for 5 minutes and adsorbed on P81 paper (Whatmann), and then the paper was washed four times with 75 mM phosphoric acid, three times with water and once with acetone. The paper was dried, and the residual radioactivity was measured using a liquid scintillation counter. The inhibitory activities of the test compounds were measured at three concentrations, 1, 10 and 100 nM, and the IC50 value was obtained from a graph depicted based on points from these three concentrations. The results of TPK1 IC_{50} values (nM) for compounds according to the present invention are shown in the attached Table-1, and the results of TPK1 IC_{50} values (nM) for Compound Nos. 38 and 39 of EP 1136482 A1 to Almario-Garcia are shown below.

No. 38



IC50 : >1000nM

No.39



IC50 : >1000nM

7. That the compounds of the present invention markedly inhibited the P-GS1 phosphorylation by TPK1, whilst Compound Nos. 38 and 39 of EP 1136482 A1 to Almario-Garcia were revealed to have much lower inhibitory activity. Compound Nos. 38 and 39 of EP 1136482 A1 to Almario-Garcia had IC50 values of more than 1,000 nM sufficiently indicating the weak inhibitory activity of the tested compounds so that further tests, including shifting to a higher concentration range, were not performed to determine exact IC50 values of Compound Nos. 38 and 39 of EP 1136482 A1 to Almario-Garcia.

8. The undersigned further declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-captioned application or any patent issuing thereon.

March 8, 2007
Date

X Kazutoshi Watanabe
Kazutoshi Watanabe